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MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST TYPE I INTERFERON

Ins D1

The interferons (IFN) constitute a group of secreted proteins which exhibit a wide range of biological activities and are characterized by their capacity to induce an antiviral state in vertebrate cells (I. Gresser and M.G. Tovey Biochem Biophys. Acta 516:231, 1978). There are three antigenic classes of IFN : alpha (α), beta (β) and gamma. IFNα and IFNβ together are known as the type I interferon.

Natural type I human interferon comprises 12 or more closely related proteins encoded by distinct genes with a high degree of structural homology (Weissmann and Weber, Prog. Nucl. Acid. Res. Mol. Biol. 33:251, 1986).

The human IFNα locus comprises two subfamilies. The first subfamily consists of 14 non allelic genes and 4 pseudogenes having at least 80% homology. The second subfamily, αII or omega (ω), contains 5 pseudogenes and 1 functional gene which exhibits 70% homology with the IFNα genes (Weissmann and Weber 1986).

The subtypes of IFNα have different specific activities but they possess the same biological spectrum (Streuli et al. PNAS-USA 78:2848, 1981) and have the same cellular receptor (Agnet M. et al. in "Interferon 5" Ed. I. Gresser p. 1-22, Academic Press, London 1983).

The interferon β (IFNβ) is encoded by a single gene which has approximately 50% homology with the IFNα genes.

The interferon α subtypes and interferon β bind to the same receptor on the cell surface.

The interferon gamma (IFN gamma) is also encoded by a single c py, which has little homology with the IFNα and IFNβ gen s. The receptor for IFN gamma is distinct from the receptor of the α and β interferons.

For the purpose of the present invention the receptor of α and β classes of IFN will be designated IFN-R. This represents natural type I receptor. The group of proteins forming natural interferon α will be designated IFNα, and type I-IFN will represent both natural IFNα, IFNω, and IFNβ.

Despite the fact that interferon is a potent antiviral agent, there is considerable evidence to suggest, that many of the characteristic symptoms of acute virus diseases such as upper respiratory tract infections are caused by an overproduction of interferon alpha. Furthermore, IFN alpha has been shown to contribute to the pathogenesis of certain chronic virus infections in experimental animals and the available evidence suggests that this is also the case for certain human chronic virus diseases such as those due to measles virus.

The interferons α are also potent immuno-regulatory molecules which stimulate polyclonal B-cell activation, enhance NK cell cytotoxicity, inhibit T-cell functions, and modulate the expression of the major histocompatibility complex (MHC) class 1 antigens, all of which are implicated in the induction of autoimmunity and in graft rejection. The abnormal production of interferon α is associated with a number of autoimmune diseases and inflammatory disorders including systemic lupus erythematosus (SLE), type I diabetes, psoriasis, rheumatoid arthritis, multiple sclerosis, Behçet's disease, aplastic anemia, the acquired immunodeficiency syndrome (AIDS) and severe

combined immunodeficiency disease. The presence of interferon α in the serum of patients with systemic lupus is correlated with both th clinical and humoral signs of increased disease activity. The production of interferon α in HIV positive subjects is also highly predictive of disease evolution.

Administration of interferon α has been reported to exacerbate underlying disease in patients with psoriasis and multiple sclerosis and to induce a SLE like syndrome in patients without a previous history of autoimmune disease. Interferon α has also been shown to induce glomerulonephritis in normal mice and to accelerate the outset of the spontaneous autoimmune disease of NZB/W mice.

Interferon α is also produced during the course of graft-versus-host disease (GVHD) in parallel with the enhanced NK cell activity characteristic of systemic GVDH. Interferon α is the principal modulator of NK cell cytotoxicity and administration of interferon α has been shown to enhance the intestinal consequences of GVDH in normal mice.

The object of the present invention is to provide new antagonists against the biological activities of the human type I-IFN. These antagonists could be used for therapeutical, including prophylaxis purposes, in cases where the type I-IFN (IFN α/β)is abnormaly produced and when this abnormal production is associated with pathological symptoms. Such antagonists could also be used for the diagnosis of various diseases or for the study of the evolution of such diseases.

In order to define such antagonists, the inventors have taken into account the fact that the human natural type I-IFN is in fact constituted of a mixture of

interferons (subspecies) and the fact that the composition of this ass ciation of different subtypes of interfer ns varies both quantitatively and qualitatively.

Some natural interferons, such as the ones secreted by Namalwa cells (Namalwa interferon) or leukocyte (leucocyte interferon) have been studied in detail (N.B. Finter and K.H. Fautes, Interferon 2, 1980, p. 65-79 I. Gresser Editor Academic Press ; K. Cantell et al, Interferon 1, 1979 p. 2-25, I. Gresser Editor Academic Press) and were used by the inventors to define natural type I interferons.

In some pathological cases, like AIDS, interferons having some special properties have been described (O.T. Preble et al, Annals of New-York Academy of Sciences p. 65-75). This interferon involved in pathological cases like AIDS nevertheless binds to the same receptor, as described above.

One object of the present invention is to provide an antagonist of the type I-IFN, which would be able to inhibit or neutralize, to a determined extent, the biological properties of the human type I-IFN, that is to say, to neutralize <u>in vivo</u> a mixture of α, β, ω subspecies.

Accordingly the inventors have defined antibodies, especially monoclonal antibodies, which have the property of being antagonists to the type I-IFN. These antibodies are directed against the human type I-IFN receptor.

The invention thus also concerns the use of the monoclonal antibodies for the preparation of pharmaceutical compositions, useful for the treatment of symptoms associated with the abnormal production of

type I-IFN. These monoclonal antibodies are also appropriate for the preparation of diagnosis reagents.

A monoclonal antibody according to the present invention is directed against the human type I-interferon receptor (IFN-R) and is characterized by the following properties :

- it recognizes the extracellular domain of the human IFN-R, and
- it has a neutralizing capacity against the biological properties of the human type I-IFN.

The ability to neutralize the biological properties of type I-IFN can be estimated as a function of the capacity of the monoclonal antibody to neutralize the antiviral activity of the type I-IFN. Such a test is relevant in order to determine whether the antibody assayed is included within the scope of the invention, although it is clear that the biological properties of type I-IFN are not limited to its antiviral properties. Detailed procedures are given in the examples in order to enable to perform such a test of the antiviral activity. The cells tested can advantageously be Daudi-cells, which affinity for the type I-IFN is well known. The main steps of such a test would consist in :

- incubating a determined concentration of human cells responsive to human type I-IFN, with human type I-IFN in the presence of a determined concentration of monoclonal antibodies to be assayed, for a time sufficient to allow the formation of a complex between the monoclonal antibodies and the IFN-R of the human cells and/or between the type I-IFN and the IFN-R of the human cells ;

- infecting the incubated cells with a determined virus, in a determined concentration,
- washing the cells,
- resuspending the cells in culture medium,
- incubating for a time sufficient to allow virus replication ;
- lysing the cells ;
- measuring the virus replication, or measuring the inhibition of the cytopathic effect.

The ability of the monoclonal antibodies of the invention to neutralize the biological properties of the human type I-IFN can be modulated as a function of the dose of antibodies used. Accordingly a 100% inhibition of the biological properties, or a partial inhibition can be obtained.

According to another embodiment of the present invention, the monoclonal antibodies directed against the human type I-IFN receptor, are further characterized by the fact that they are capable of inhibiting the binding of a human type I-IFN, to the human IFN-R.

A monoclonal antibody having the capacity to recognize the extracellar domain of the human IFN-R and capable of inhibiting the binding of the human type I-IFN to its receptor, can be selected by the following steps :

- preincubating a determined concentration of purified monoclonal antibodies or a hybridoma culture supernatant containing monoclonal antibodies to be assayed, with human cells capable of harboring IFN-R ;
- adding labelled human type I-IFN, in a determined concentration, to the above preincubated medium ;

- incubating the medium containing th human cells, the monoclonal antibodies and the labelled type I-IFN for a time sufficient to allow an equilibrium to occur, between the monoclonal antibodies on the one hand and the type I-IFN on the other hand, with the cellular IFN-R ;
- washing the cells ;
- determining the formation of a binding complex between the human cells and the labelled type I-IFN by counting the amount of attached labelled type I-IFN.

Some of the monoclonal antibodies of the invention, have also the capacity to neutralize the antiproliferative properties of the human type I-IFN. This property can also be assayed on Daudi cells, by performing the following steps :

- allowing cells to grow in presence of human type IFN and determined concentration of mAb ;
- counting the cells in order to detect an inhibition of the antiproliferative effect of the human type I-IFN.

One property of a monocolonal antibody according to the invention resides in its capacity to recognize the extracellular domain of the human IFN receptor. This property of the monoclonal antibody can be assayed on human cells bearing the natural human receptor but also on the extracellular domain of a recombinant IFN-R such as expressed in a procaryotic cell, for instance in <u>E.coli</u> or a recombinant IFN-R such as expressed in a eucaryotic cell such as mamalian cell for instance a CHO-cell.

This receptor can indeed present different properties, depending on the fact that it is produced in a procaryotic or eucaryotic cell and accordingly

depending on the fact that the post-translational maturation occurred or not. The inventors interestingly showed that relevant assays, to evaluate the capacity of a monoclonal antibody according to the invention i.e. to recognize the cellular IFN-R, can be performed on a recombinant receptor expressed in mamalian cells. As a matter of fact, such recombinant receptor has the same properties as the cellular receptor, as far as its recognizing activity is concerned.

Monoclonal antibodies of the invention can be obtained against various forms of the receptor, including the complete receptor, a particular domain or a peptide characteristic of the aminoacid sequence of the receptor represented in figure 3 (SEQ ID NOS: 3-4)

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Monoclonal antibodies of the invention ~~can~~ for example be prepared against the ~~soluble~~ form of the receptor. A hydrosoluble polypeptide corresponding to the soluble form of the INF-R is described on figure 2 (SEQ ID NOS: 1-2). According to the present invention, a soluble form of the IFN-R corresponds to a peptide or a polypeptide, capable of circulating in the body.

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Other monoclonal antibodies according to ~~the~~ invention can also be prepared against ~~a~~ peptide comprised in the extracellular domain of the receptor as described on figure 2 SEQ ID NO: 1 or 2. ~~An~~ advantageous peptide corresponds for instance to the aminoacid sequence comprised between aminoacid 1 and aminoacid 229 SEQ ID NO: 1 or 2. According to another embodiment of the invention, the antibodies can be prepared against a polypeptide modified by substitution of one or more amino acids, provided that antibodies directed against the non modified extracellular domain of the IFN-R, recognize the modified polypeptide or peptide.

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aa seq is 4 not 3

aa seq is 2 not 1

Preferred mon clonal antibodies according to the invention are those which are of the IgG1 type.

Among th antibodies of the invention, an antibody which has the capacity of inhibiting the binding of the type I-IFN to its receptor is preferably characterized in that it inhibits the in vitro binding of human type IFN, to the human cellular IFN-R when it is co-incubated with cells harboring the hu-IFN-R, at a concentration of antibodies equal or inferior to 100 µg/ml, preferably equal or inferior to 50 µg/ml, advantageously inferior to 20 µg/ml, more preferably in the range of approximately 0.5 to 2 µg/ml.

The inventors have shown that the high affinity binding capacity of a monoclonal antibody is not sufficient to ensure that this antibody will be able to inhibit the binding activity of the human type I-IFN to the IFN-R. Nevertheless the high affinity binding capacity of the monoclonal antibody is necessary to investigate further the ability of the antibody to inhibit the binding of the type I-IFN to its cellular receptor.

Another monoclonal antibody is characterized in that it neutralizes in vitro the antiproliferative activity of human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 10 µg/ml.

According to another embodiment a monoclonal antibody is also characterized in that it neutralizes in vitro the antiproliferative activity of human typ IFN, on cells poorly responsive to this human IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 µg/ml.

A particular group of monoclonal antibodi s according to the invention is characterized in that it

neutralizes the antiviral activity of the human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 50 µg/ml, preferably 1 to 20 µg/ml, for a concentration of type I-IFN in the range of 1 to 1000 units with reference to the international standard MRC 69/19.

Advantageously, the monoclonal antibody according to the invention is such that these antibodies do not bind to the human receptor for IFN gamma.

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One particular antibody ~~satisfying the requirements of the invention, is~~ such as it is directed against an epitope ~~on the~~ amino-acid sequence comprised between amino-acid 27 and amino-acid 427 of the extracellular domain of the human IFN-R as represented ~~on figure 2~~ SEQ ID NOS: 1-2.

One particularly interesting monoclonal antibody according to the invention is the antibody designated 64G12 under n° 92022605 which has been deposited at the ECACC (European Collection of Animal Cell Cultures Porton Down Salisbury, Wiltshire SP4 056, United Kingdom) on February 26, 1992.

These antibodies may be prepared by conventional methods involving the preparation of hybridoma cells by the fusion of myeloma cells and spleen cells of an animal immunized beforehand with the peptide antigen, on the conditions such that the antigen against which the antibodies are formed is constituted by the extracellular domain of IFN-R or any polypeptide or peptide of this domain.

The hybridomas are constructed according to the protocole of Kohler and Milstein (Nature, 1974, 256: 495-497). For example the hybridomas are derived from

the fusion of the spleen cells above described with NS1 m use (BalbC) HGPRT$^-$ as myeloma cell.

A second procedure for the production of monoclonal antibodies according to the invention, consists in carrying out the fusion between B-cells of blood immortalized with the Epstein/Barr virus and human B lymphocytes placed beforehand in contact with the extracellular domain or a fragment thereof of the IFN-R, against which it is decided to form monoclonal antibodies. B-cells placed in contact beforehand with the extracellular domain of IFN-R or fragment thereof against which it is decided to form monoclonal antibodies, may be obtained by in vitro culture contacted with the antigens, the recovery of the B-cells coated with these antigens being preceded by one or several cycles of stimulation.

The invention thus concerns human antibodies as obtained by carrying out the above procedure, having the above defined properties.

The invention also aims at providing a monoclonal antibody characterized in that the variable or complementary determining regions of its heavy and/ r light chains are grafted on the framework and/or constant regions of a human antibody.

The invention further provides a compositi n having antagonist properties for the biological properties of the human type I-IFN, characterized in that it comprises monoclonal antibodies as defined above.

Accordingly the invention provides a pharmaceutical composition characterized in that it comprises monoclonal antobodies as defined above, together with an appropriate pharmaceutical vehicle.

The inv ntion also concerns th use of a monoclonal antibody as defined above, for the manufactur of a drug for the treatment or profilaxis of a pathological state or symptoms associated with overproduction of type-I-IFN.

According to a first example, the antibodies can be used in a pharmaceutical composition, for the treatment of allograft rejection.

According to another example, antibodies of the invention are used as active principle in a pharmaceutical composition for the treatment of autoimmune and inflammatory diseases. Such diseases include systemic lupus erythematosus, type 1 diabetes, psoriasis, rheumatoid arthritis, multiple sclerosis, Behçet's disease, asplatic anemia, acquired immunodeficiency syndrome (AIDS), and severe combined immunodeficiency disease.

Treatment of acute virus diseases can also be performed with the antibodies of the invention. As example upper respiratory tract infections, chronic virus infections such as those due to measles virus, can be performed.

The antibodies of the invention can also be used for the <u>in vitro</u> diagnosis of the presence of the human type I-IFN receptor or cells.

Further details and additional information will arise from the description from the description of the examples and from the figures.

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~~FIGURES~~ BRIEF DESCRIPTION OF THE DRAWINGS:

- Figure 1 : binding of ^{125}I-labelled monoclonal antibodies 34F10 and 64G12 to :
  - A : Daudi cells
  - B : Ly28 cells

Briefly, 10^6 cells were incubated for 2 hours at 4°C in presence of different amounts of the labelled antibodies diluted in RPMI medium containing 10% fetal calf serum (FCS). The cells were then washed 4 times in RPMI-1% FCS and counted for bound radioactivity. Nonspecific binding was mesured by incubation with a 100 fold exces of cold antibodies and substracted from total counts.

- Figure 2A and 2B: nucleotide and corresponding amino-acid sequence (SEQ ID NOS: 1-2) of the extracellular domain of the human IFN-R

The monoclonal antibodies were produced against recombinant soluble forms of the human interferon alpha-beta receptor (IFN-R) synthetized in either procaryotic cells (E.coli) or a mammalian cell system (Cos cell). These soluble forms were based on the DNA sequence described in figure 2.

- Figure 3A and 3B : nucleotide and corresponding amino-acid sequence (SEQ ID NOS: 3-4) of the human IFN-R.

EXAMPLES

EXAMPLE 1 :

Synthesis of the soluble receptors

Synthesis in E.coli

A fragment of DNA containing the sequence coding for the extracellular domain (amino acids 27 to 427) of the human INF-R (figure 2), SEQ ID NOS: 1-2 in which an extra-sequence coding for 5 histidyl residues was introduced just before the termination codon, was cloned in the expression vectors pKK233-2. This fragment was produced by the Polymerase Chain Reaction (PCR) and the resulting plasmids were sequenced to confirm both in-frame insertion with the Shine-Dalgarno sequence and the appropriate sequence coding for the receptor.

The poly-histidyl tail introduced into the recombinant protein enables it to be purified rapidly by affinity chromatography on a chelated nickel support (NTA column) as described previously (Hochuli E. et al, Bio/technology, 1988, 1321-1325).

The plasmid was introduced into the E.coli strain, JM105, and protein synthesis induced by addition of IPTG to the culture medium (pKK233-2, tac promoter).

Proteins were extracted from the bacterial pellet and the soluble receptor purified to homogeneity by affinity chromatography as described hereafter. This procedure yieled a protein that migrates as 2 bands around 50 kDa under reducing conditions and three bands under non-reducing conditions. The maximum concentration of the protein obtained by different procedures was approximately 20µg/ml.

The N-terminal sequence of the two proteins detected by gel el ctrophoresis has shown that both proteins are the expected fragment of the receptor.

<u>Synthesis and purification of an unglycosylated soluble receptor :</u>

Bacterial culture (250ml)

|

IPTG induction 3h

|

cell pellet
6M Guanidine hydrochloride pH8

|

centrifugation

|

NTA column: Washes pH 8 urea 8M
pH 6,3 urea 8M
pH 5.9 urea 8M

|

Elution pH 4 urea 8M

|

refolding dilution, dialysis
against Tris 0,1 M pH9

|

dialysis PBS

Using the same PCR approach, we also constructed an expression vector coding for the IFN-R amino acid sequence 1-427, with an additional 5-histidyl residues at the C-terminus, inserted in expression vector pXMT-3. The exact nucleotide sequence of the insert was also confirmed.

The resulting plasmid was introduced by electroporation into Cos7 cells for transient expression and the recombinant protein was purified to homogeneity by affinity chromotography followed by ion exchange chromatography on mono-Q (Pharmacia) as described hereafter.

Purification of the soluble IFN-R from Cos7 cells

preparative electroporation of cos cells

18 h

serum free medium

supernatants taken after 48h, 72h, 96h

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concentration

NTA column

Wash PBS

elution 0.1 M NaOAc pH 5.5

neutralization

concentration, 30 000 cut off

Mono Q (0-0.5 M Na Cl)

MONO Q

This purification yielded to a 76 kDa protein whose N-terminal sequence corresponds to the predicted receptor sequence with some heterogeneity in the processing of the leader sequence.

EXAMPLE 2 :

Production of monoclonal antibodies against the interferon type I receptor

1) Production of the monoclonal antibodies

Mice were immunized by injection of recombinant soluble interferon (r sIFN-R) purified from E.coli or from a culture supernatant of Cos7 cells. Initially mice were injected both intraperitoneally and subcutaneously with the purified protein in complete Freund's adjuvant. Subsequently mice were injected once a week intraperitoneally with the purified proteins diluted in buffered saline solution. Ten micrograms of recombinant proteins were injected each time.

After the fourth injection, blood was collected and the presence of specific serum antibodies were tested by both ELISA and Western blot against the recombinant receptor. The strongest responders were then boosted with a total of 10μg of antigen half of which was injected intravenously and half intraperitoneally.

2) Cell fusion

Four days after boosting, spleen cells from the immunized animal were collected and fused to NS1 (mouse) (Balbc) HGPRT$^-$ myeloma cells according to the method described by S. Fazekas et al. (J. Immunol. Methods 35:1-32, 1980). Briefly, 5×10^7 spleen cells were fused to 3×10^7 myeloma cells in 1ml of polyethylene glycol solution and distributed in five 96 well plates on a peritoneal macrophage feeder layer in HAT (hypoxanthine, aminoprotein and thymidine) medium. This procedure was repeated 4 times as 20×10^7 spleen cells were obtained from the immunized mouse. Screening for specific hybridomas was undertaken when large colonies were detectable in culture wells.

For the screening, presence of specific antibodies was determined by a direct ELISA method :

a) ELISA plates were coated overnight at 4°C with purified E.coli-expressed or Cos7 cell-expressed sIFN-R diluted in PBS. Plates coated with BSA were used to detect non specific binding,

b) Plates were saturated by incubation with 3% BSA in PBS for 1 hour at 37°C,

c) Plates were incubated for 4 hours at room temperature with hybridoma supernatants diluted 1 in 4 with PBS-0.05% Tween 20, TWEEN 20

d) Bound antibodies were detected by a two step procedure, comprising a first incubation with goat anti-mouse biotinylated immunoglobulin followed by streptavidin-horseradish peroxidase complex (both from Amersham and diluted 1/1000 in PBS-0.05% Tween 20). TWEEN 20.

Positive antibody secreting hybridomas were passaged in 24 well plates on a spleen cell feeder layer and their reactivity was again checked by ELISA, and Western-blot.

3) Identification of reactivity to the natural interferon type I receptor

The reactivity of the monoclonal antibodies (mAbs) recognizing the recombinant sIFN-R was tested against the natural class I receptor expressed at the surface of Daudi cells, by membrane immunofluorescence. Briefly, $5x10^5$ Daudi cells were incubated in 100μl of culture supernatant of chosen hybridomas for 30 min at 4°C. The cells were then washed 4 times in RPMI medium containing 1% BSA and further incubated with a diluted FITC labelled goat anti-mouse $F(ab')_2$ for 30 min at 4°C. The cells were finally analyzed by flow cytometry after washing. One of the 35 tested antibodies produced against the E.coli recombinant receptor and 5 of the 6 tested antibodies produced against the COS recombinant receptor were found to recognize the natural receptor on the Daudi cells.

Cloning of these hybridomas was then performed by limiting dilution. The isotype of these mAbs was determined by an ELISA method using isotype specific antibodies. All 6 mAbs were found to be IgG1 with kappa light chains. A summary of the reactivity of these 6 mAbs is given in Table 1.

Monoclonal antibodies were purified from culture supernatants by protein G chromatography.

Table 1 :

Reactivity of th anti IFN-R monocl nal antib dies

	Reactivity against the recombinant receptor				Reactivity against * the cellular receptor
	E.COLI 22-422		COS 1-422		
	ELISA	Western	ELISA	Western	immunofluorescence
34F10	+	+	+	+	+
64G12	+	+	+	+	+
63F6 64G2 64D10 65D8	-	-	+	+ weak	+

* measured on Daudi cells

EXAMPLE 3 :

Inhibition of the binding of interferon to human cell lines

Inhibition of interferon binding to human cells was assayed as follows. 10^6 cells were preincubated at 4°C for 30 min with various dilutions of hybridoma culture supernatants or purified mAbs or with medium alone. ^{125}I-labelled IFN alpha 8 or alpha 2 was added at the concentration of 100pM and cells incubated for a further 2 hours at 4°C. These incubations were performed in RPMI medium containing 20mM HEPES pH 7.4 and 10% foetal calf serum (FCS). The cells were finally washed 4 times with RPMI - 1% FCS and counted to determine bound radioactivity.

The mAb secreted by _th hybridoma line 64G12 (latter named mAb 64G12) was shown in this assay to inhibit the binding of labelled IFN to the cells in a dose-dependent manner. 50% inhibition of binding to the Daudi cells (Burkitt lymphoma cell line ; Klein et al., Cancer Researh, 28:1300-1310, 1968) was obtained at a mAb concentration of 0.4μg/ml. The same inhibition was obtained with K562 cells (chronic myelogenous leukemia, Lozzio and Lozzio, Cell, 45:321-334, 1975) but 50% inhibition was obtained at 11μg/ml for HL60 cells (Promyelocytic leukemia, Collins S.J. et al., Nature, 270:347-349, 1977) and 60μg/ml for Ly28 cells (Klein G. et al. Int. J. Cancer, 10:44-57, 1972).

Table 2 :

The inhibition of binding of labelled IFN alpha 2 to various cell lines by mAB64G12

Cell lines	Concentration of mAB which gives 50% inhibition of binding
Daudi K562	0,4 μg/ml
HL60	11 μg/ml
Ly28	60 μg/ml

The difference in the mAb concentration at which 50% inhibition of binding of IFN is obtained has been investigated by direct binding of ^{125}I-labelled mABs 64G12 and 34F10 to the same cell lines and Scatchard

plot analysis of the results. In the concentration range of 0.1 to 1.5 µg/ml, a high affinity binding of the mAb 34F10 (≈10nM) was seen on all cell lines whereas a high affinity binding of mAB 64G12 was only detected on Daudi and K562 cells (Figure 1).

EXAMPLE 4 :

Inhibition of the function of type I interferon

Functional inhibition of type I interferon by the purified mAb 64G12 was demonstrated in an antiviral assay on Daudi cells using either recombinant IFN alpha 2, IFN beta and IFN omega, or purified Namalwa and leucocyte interferons, and in an antiproliferative assay with recombinant IFN alpha 2.

* Antiviral activity

An antiviral assay on Daudi cells was performed as described (M. Dron and M.G. Tovey, J. Gen. Virol. 64:2641-2647, 1983). Cells (0.5×10^6/ml) were incubated for 24 hours in the presence of interferon and antibodies. 10^6 cells in 1 ml were then infected for 1 hour at 37°C with Vesicular stomatitis virus (VSV) then washed 3 times, resuspended in culture medium and incubated for 18 hours at 37°C. Cells were then lysed by freeze-thawing and virus replication measured by titration of the supernatants on L929 cells. A dose-dependent inhibition of the antiviral activity of the various subtypes of type I IFN was demonstrated for the purified mAb 64G12.

For the antiviral assay with the Wish cells, cells were incubated for 24 h urs with various concentrations of interfer ns in the presence f the mAbs prior to challenge with VSV. In this assay, the mAb 64G12 was demonstrated to block completely the antiviral activity of Leukocyte IFN (50U/ml), recombinant IFN alpha 2 (50U/ml) and interferon from the sera of AIDS patients (50, 75 and 150U/ml).

* antiproliferative activity

For the antiproliferative assay, Daudi cells were seeded at a concentration of 10^5 cells per ml in a 96 well plate in the presence of interferon and purified inhibitory or control antibody. Cells were then counted after 24, 48 and 72 hours with a Coulter counter and checked for viability by trypan blue exclusion. Purified mAb 64G12 demonstrated a dose-dependent inhibition of the antiproliferative activity of interferon alpha 2.

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